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Ultrastructure of the dermal chromatophores in the Fringe-toed lizard, *Acanthodactylus orientalis*

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ABSTRACT. Histology and electron microscopic studies of the dorsal skin of the Fringe-toed lizard, *Acanthodactylus orientalis* Angel, 1936, showed three types of dermal chromatophores: xanthophores, iridophores and melanophores. These pigment cells were observed in vertical combination, with an uppermost layer of xanthophores, an intermediate layer of iridophores and a basal layer of melanophores. The ultrastructure of the melanophore is characterized by oval nucleus and numerous pigment granules, the melanosomes of different stages that remain scattered in the cytoplasm. The chromatophores of this species contain significant information of anatomical similarity with lower as well as higher vertebrates. They can help to better understand the inter relationships between vertebrate pigment cells and their role in skin dysfunctions.

KEY WORDS. Histology, Xanthophores, Iridophores, Melanosomes, anatomical significance.

INTRODUCTION

The stratifying, multilayered epidermis that forms the outermost layer of the skin of all vertebrates is a barrier protecting the body from abrasion, dehydration and microbial infections (Alibardi and Toni 2006, Alibardi 2006, Koster and Roop 2007, Proksch et al. 2008, Lillywhite et al. 2009). The pigmentation on the integument of animals forms one of the most dramatic and conspicuous biological patterns. The epidermis of lepidosaurians (lizards, snakes, and sphenodontids) is of particular complexity and interest (Hildebrand and Goslow 2001). It has very colorful scales and is organized in an attractive pattern with hard (beta) and soft (alpha) layers (Chang et al. 2009). A scaly, keratinized integument is one of the characteristic features that airproof the skin of reptiles (Hildebrand and Goslow 2001).

In the dermal skin of reptiles four basic types of pigment cells have been recognized: xanthophores, erythrophores, iridophores, and melanophores (Cooper and Greenberg 1992, Bagnara 1998, Kuriyama et al. 2006). The color intensity as well as the patterns of the skin in different reptilian species vary according to the distribution of epidermal melanocytes and dermal melanophores, lipophores (xantophores and erythrophores), and iridophores (Szabo et al. 1973, Bagnara 1983, Gosner 1989, Cooper and Greenberg 1992, Macedonia et al. 2000, Bagnara and Matsumoto 2006, Alibardi 2011, 2012, 2013). The spatial arrangement and architectural combination of these pigment cells can produce a multitude of skin colors in reptiles (Morrison 1995, Morrison et al. 1996, Kuriyama et al. 2006). In the dermis, xantophores and erythrophores (light-absorbing pigment cells) contain xantophils, carotenoids, and pterins and produce orange-yellow to reddish colors (Ferrer et al. 1999, Steffen and McGraw 2009). Iridophores contain light-reflecting platelets made of crystalline purines and pteridines, which give rise to structural colors by thin-layer interference and the scatter or diffraction of light from the stacks of reflecting platelets (Bagnara 1998). The size, shape, orientation, number, and conformation of reflecting platelets and the distance between them determine what structural colors the iridophores produce (Morrison 1995, Morrison et al. 1995, 1996). Melanophores are light-absorbing pigment cells that absorb light to produce black or brown colors.

Earlier ultrastructural studies on the melanophores of various classes of vertebrates such as teleosts and lung fishes (Fujii and Novales 1969, Imaki and Chavin 1975a,b), reptiles (Roth and Jones 1967) and larval amphibians (Wise 1969) have shown the remarkably consistent fine structural features of these melanosomes while they are synthesizing and containing cells, and explained their role in physiological color changes. However, the pattern of distribution and association of these cell types has been found to vary considerably in different vertebrates (Imaki and Chavin 1975a). In most reptiles, lizards, sphenodon, snakes, turtles, and crocodilians, abrupt color variation does not occur, only a darkening or lightening effect during periods of weeks

or months is observed (Sherbrooke and Frost 1989, Roweet al. 2006). In order to understand the histological basis of color patterns in certain lizard species, the ultrastructure of chromatophores must be characterized and the structural combinations of each type of chromatophore in skin layers must be determined.

Despite the body of literature available on melanophores, there have been no studies on the fine structure of the dorsal skin chromatophores of the fringe-toed lizard, *Acanthodactylus orientalis* Angel, 1936. This species is commonly known from central and southern Syria, northern Jordan and western and central Iraq (IUCN 2015). Hence, the present study was carried out to investigate in detail the fine structure of the dorsal skin chromatophores of *A. orientalis* by means of light and transmis sion electron microscopy, to provide a connecting link between melanophore structure and function in different reptilian species.

MATERIAL AND METHODS

Eleven specimens (average SVL = 8.25 cm; average weight 9.02 g) of *A. orientalis* were captured by the noosing method during the spring of 2014, in the northern part of the region of Turaif (31°40'39"N 38°39'11"E), Kingdom of Saudi Arabia. Each specimen was measured to record SVL to the nearest 0.1 mm and weighed to the nearest 0.1 g. The captured lizards were transported to the Reptilian laboratory of the Zoology Department, College of Science, King Saud University, where all experimental procedures were performed. Animals were kept at ambient temperature (23 ± 1.5 °C) and with natural photoperiod i.e. 12 hours of light-dark cycle. All field data such as locations of the lizards and their altitude were recorded. All animals were euthanized in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the King Saud University, Riyadh; Kingdom of Saudi Arabia.

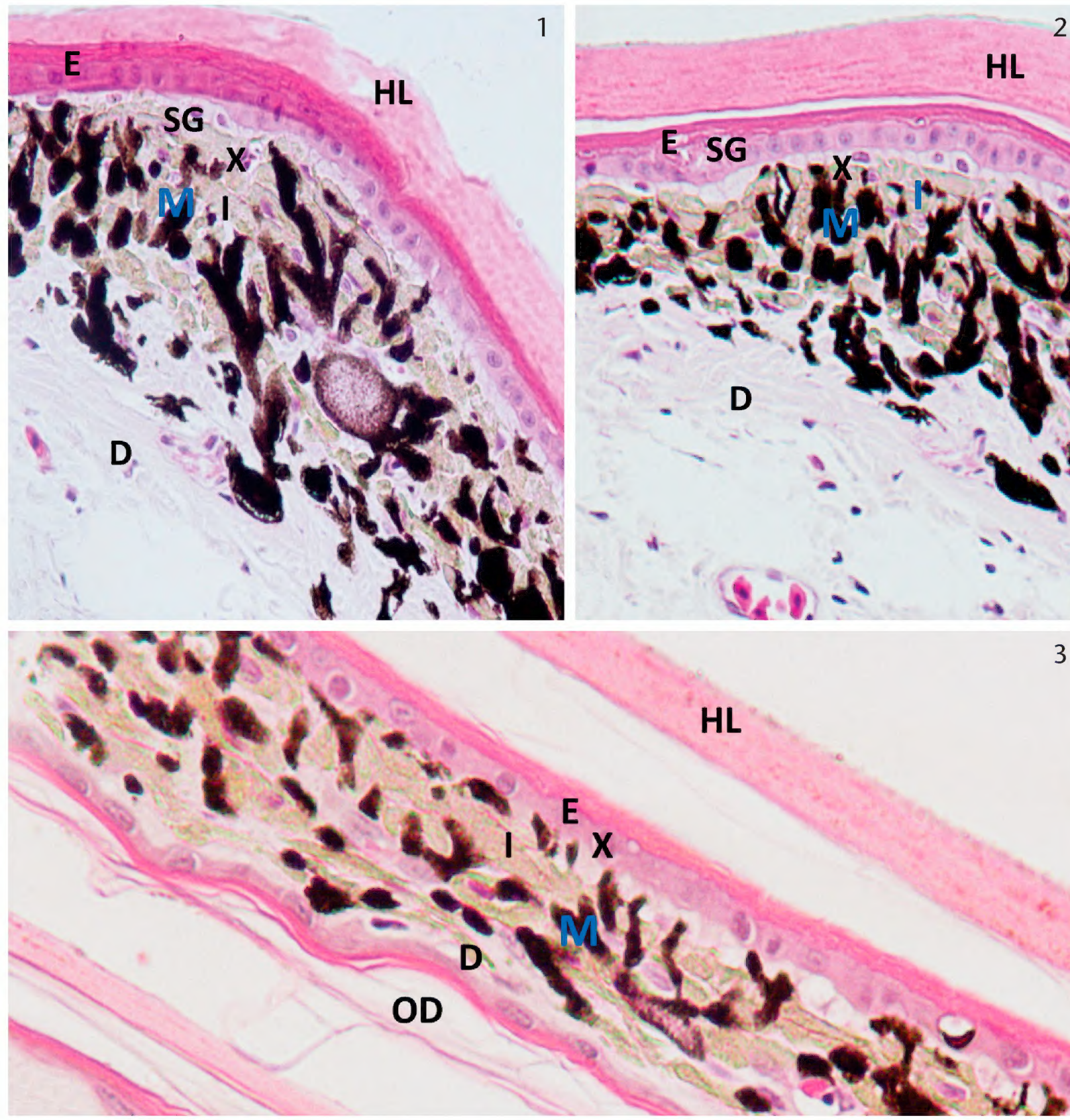
For the histological studies the dorsal skin of animals were quickly removed from the trunk region with a sharpened steel scissor under ethyl ether anesthesia and fixed in 10% neutral buffered formalin for 72 hours. The fixed specimens were processed overnight for dehydration, clearing and impregnation using an automatic tissue processor (Sakura, Japan). The specimens were embedded in paraffin blocks using embedding station (Sakura, Japan) and sections of 4 µm thickness were cut using rotary microtome (Leica-RM2245, Germany) and an Autostainer (Leica5020, Germany) was used for Hematoxylin and Eosin staining. The stained sections were observed under light microscopy Eclipse BOi (Nikon, Japan) and the images were taken with digital microscopic mounted camera (OMX1200C Nikon, Japan).

For Transmission electron microscopic studies, 2 x 4 mm skin samples were collected from the dorsal region of animals for microscopic observation. The skin samples were immediately fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) overnight at 4 °C. The tissues were rinsed with 0.25 M sucrose in 0.1 M phosphate buffer (pH 7.3) and were then fixed in 0.1 M buffer containing 1% osmium tetraoxide (OsO_4) for 1.30 hours at room temperature. Dehydration of the fixed tissue was performed using increasing concentrations of ethanol. The specimens were substituted in propylene oxide before embedding in pure resin (SPI, Toronto; Canada) (Reynolds 1963). Ultra-thin sections (2–4 µm) were cut with a diamond knife (Biel, Switzerland) on anultra-microtome (Leica, UCT; Germany). Ultra-thin sections were then placed on copper grids and double stained with uranyl acetate (20 minutes) and alkaline lead citrate (5 minutes). The electron micrographs were produced with a JEOL JEM-1011transmission electron microscope at 80 kV and Gathan software at the Research Center, King Saud University, Riyadh, Saudi Arabia.

RESULTS

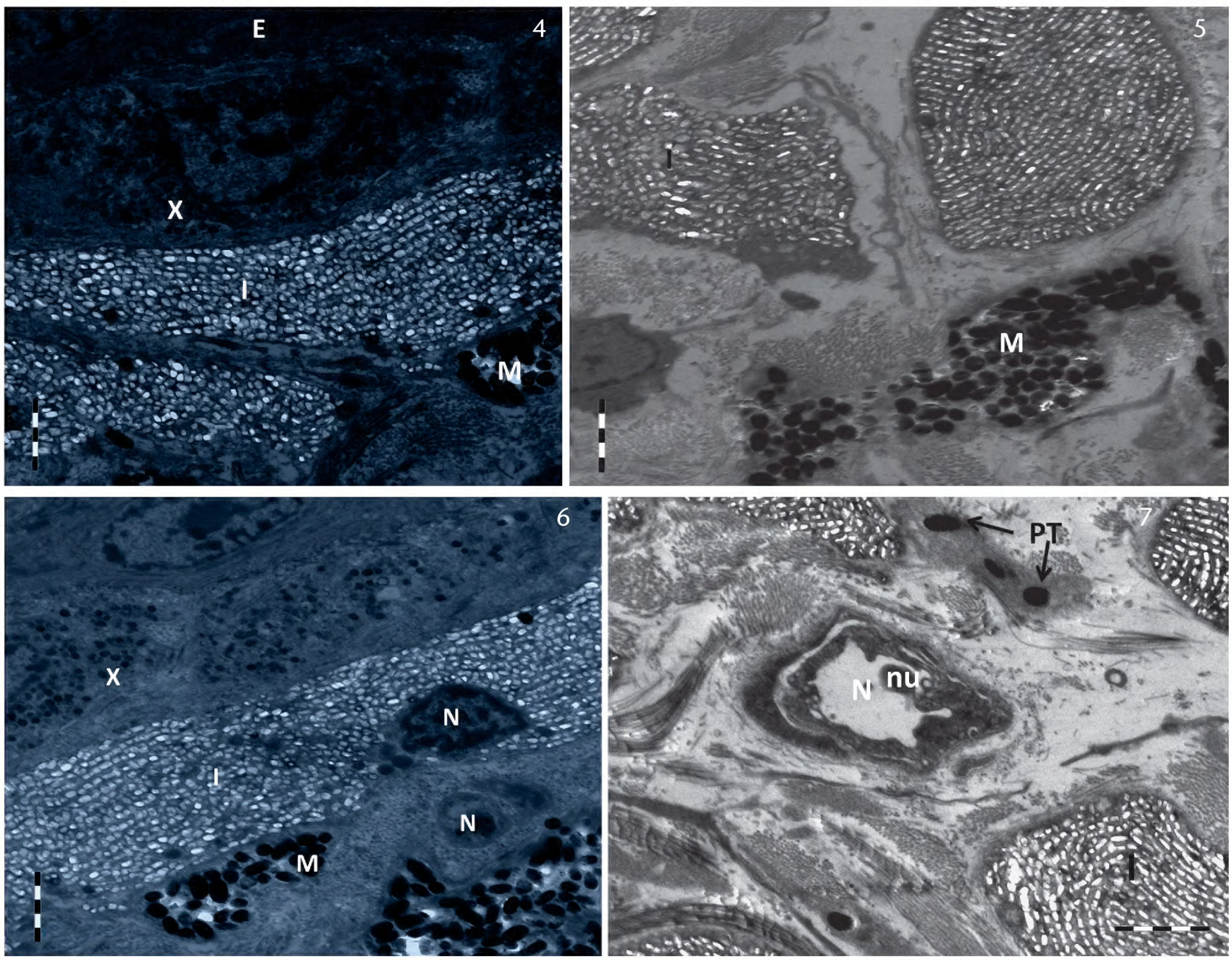
The clear epidermal and dermal layers showed the general reptile skin structure in cross sections of fixed tissue, under the light and the transmission electron microscopes. The epidermal layer consisted of the outer epidermal generation and the stratum germinativum, in cross sections of the dorsal skin when observed under the light microscope (Fig. 1). The horny epidermis (beta layer) forms the outermost layer in the epidermal generation, which is followed by the cells of the intermediate zone (thick mesos layer) transiting underneath into thicker cells, forming an incomplete alpha-layer. The basal cell layer, stratum germinativum, is the base of the epidermis, from which the upper epidermis is generated during ecdysis. The dermal layer seen beneath the epidermis contains chromatophores and the bony osteoderm filled with collagen fibers. The beta-layer appeared dark from the accumulation of pigments and abundant dermal melanophores were seen beneath the epidermis around the dark areas (Fig. 2). These melanophores were seen scattered in the dermis, mostly xanthophores are outermost in position and overlie iridophores (reflecting cells) which in turn are above the melanophores (Figs 1–3).

Electron microscopic observations of the chromatophores of *Acanthodactylus* confirm earlier descriptions from light microscopy. In *A. orientalis* with four pairs of dark gray longitudinal strips on a beige to sandy reddish background of the trunk, epidermal melanophores and three types of dermal chromatophores (xanthophores, iridophores and melanophores) were observed under TEM (Figs 4, 5). Iridophores, seen clearly in ultra-thin secions of fixed tissue observed byTEM, were similar in ultrastructure to those described for other ectothermic vertebrates (Fig. 4), containing their specific intracellular organelle, the reflecting platelet. These reflecting platelets showed a notable regularity of position within the uniform sized iridophores, and are arranged in rows parallel to each other. These cells were detected in dark edged light spots of the trunk skin. Xanthophores were located at low frequency in the uppermost layer of the dermis in sandy reddish and brown skin above the iridophores containing their characteristic pigmentary organelles, pterinosomes (Figs 6, 7). Iridophores were detected in the dark gray skin of the trunk, but not in the trunk skin with black coloration.



Figures 1–3. Histological structure of dorsal skin in *A. orientalis*. The chromatophore layer is located just below the basal cell layer in the epidermis. (HL) Horny epidermal layer, (E) epidermis, (SG) stratum germinativum, (I) iridophore, (M) melanophore, (X) xanthophore, (D) dermis, (OD) osteoderm.

Epidermal melanophores with a nucleus at the center were detected by TEM (Figs 8, 9), located in the stratum germinativum of the epidermis. These melanophores contained highly electron-dense oval granules and looked fully melanized. Immature, unmelanized premelanosome with intraluminal fibrils were also observed. Dermal melanophores were clearly recognized and their shape was dendritic (like iridophores) in fresh tissue observed under the light microscope. They were characterized by their specific intracellular organelle, the melanosome. Melanosomes of dermal melanophores were slightly larger than those of epidermal melanophores. The skin sections showed numerous cytoplasmic elongations that were filled with electron-dense melanosomes almost reaching the basement membrane (Figs 6, 7). Dark melanosomes were similar in dermal and epidermal chromatophores in shape, from roundish to oval. Melanocytes were common in these areas. Round to oval shaped melanosomes of dermal melanophores are similar in size to epidermal melanophores. Dermal melanophores were found below the other chromatophores, such as xanthophores and iridophores, suggesting that the dendrites of melanophores extended horizontally but not vertically. Bundles of collagen fibers were also noticed.

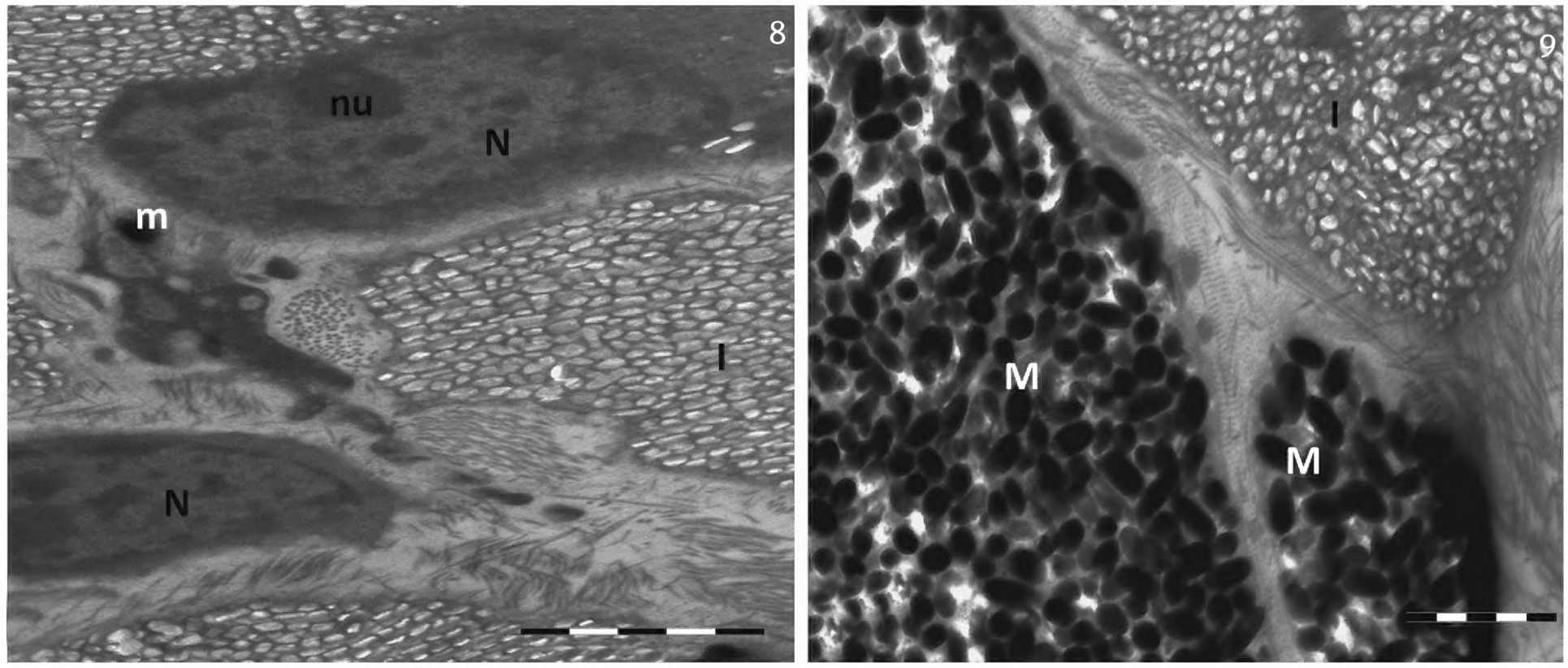


Figures 4–7. (4-5) Ultrastructural features of chromatophores of dorsal skin in *A. orientalis*. The vertical combination of dermal chromatophores is xanthophores at the top, iridophores in the middle, and melanophores at the bottom. (6-7) Electron photomicrograph showing the combination of dermal chromatophores in the skin of *A. orientalis*. (E) Epidermal layer, (I) iridophores, (M) melanophores, (nu) nucleolus, (N) nucleus, (PT) pterinosomes, (X) xanthophores. Scale bar: 2 µm.

DISCUSSION

Complex skin pigmentation patterns are exhibited by various vertebrate animals. The distribution of skin pigments is the main factor determining the ultimate pigmentation pattern of a species (Bagnara and Hadley 1973). The current study shows that in *A. orientalis,* the three typical chromatophores are not organized in a functional chromatophoric unit, as it is the case with other species of reptiles and amphibians (Bagnara and Matsumoto 2006). Many studies have been carried out to explain the location and mechanism of pigment pattern inside the melanophores of vertebrates, trying to understand the mys terious phenomenon of physiological color changes. However, it appears that even after occupying their final destinations, the melanophores retain a high degree of flexibility. In this series of events, the present investigation throws some light on the ultrastructure of the dorsal skin chromatophores of *A. orientalis*.

Under the light microscope it was observed that the melanophores of *A. orientalis* are located just below the epidermis, while some melanophores were found scattered in the dermal matrix of the skin. This finding was confirmed by electron microscope. Ultrastructural observations of the epidermal melanophores of this species also revealed the presence of a prominent oval nucleus, consistent with the findings of Ali and Naaz (2014) for the Indian toad, *Bufo melanostictus* Schneider, 1799. Melanosomes of varying degree of pigmentation were found in the cytoplasm of the melanophore around the nucleus. Mitochondria, vacuolar endoplasmic reticulum and



Figures 8–9. Electron photomicrograph showing different stages of melanosomes. (I) Iridophores, (M) melanophores, (m) mitochondria, (N) nucleus, (nu) nucleolus. Scale bar: 2 µm.

Golgi apparatus were also observed in the cytoplasm. Finding spherical pre-melanosomes near the Golgi apparatus of *A. orientalis* species confirmed the findings of Seui et al. (1961), who suggested that Golgi vesicles are possible precursors of melanosomes. The immature and developing melanosomes were also observed in the melanophores of this species as described by Fitzpatrick et al. (1965) in mammalian melanocytes and Ali and Naaz (2014) in *B. melanostictus*. A Bulk of premelanosomes of different stages was found in sub epidermal melanophores of *A. orientalis,* indicating that the active melanogenesis process occurs in the subepidermis. In comparison to dermal melanophores, pre-melanosomes occurred abundantly in the subepidermal melanophores of this species, consistent with the ultrastructural arrangement of *Neoceratodus forsteri* (Krefft, 1870) (Imaki and Chavin 1975a,b).

Electron microscopic studies of the chromatophores of *A. orientalis* confirm the scattered combination of dermalchromatophores with the uppermost layer of xanthophores, the intermediate layer with iridophores and melanophores in the basal layer. This is in agreement with the findings of Kuriyama et al. (2006) for *Plestiodon latiscutatus* Hallowell, 1861. However, there are some structural, functional and distributional differences in the iridophores. A number of layers of these reflecting cells are present above the melanophores. In addition, melanophores terminate above the xanthophores in *A. orientalis*.

The present findings throw some light on the morphoanatomic and phylogenetic details of reptilian melanophores. Here, we conclude that studies of the ultrastructure of the dorsal skin melanophores of *A. orientalis* resemble the condition found in higher vertebrates, including humans. No significant differences were observed. In conjugation with earlier studies, the present data on the ultrastructure of pigment cells continue to suggest that the process of melanin biogenesis is associated with different phases of melanosome development.

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